

INTERMEDIATE-WINGED APHIDS AND THE
TIME-OF-DETERMINATION THEORY

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Transplantation experiments by Spemann (1927), Mangold (1928), and many others have shown that the destinies of different parts of vertebrate embryos are fixed at different times in the embryogeny. The same technique is not readily open to the investigator of invertebrate development, but it has been assumed that there is a similar succession of embryonic segregations in these groups. One important genetic theory, that of Goldschmidt (1931), concerning the nature of intersexes, states that they begin development in the manner characteristic of one sex, that a physiological change constituting a turning point occurs later, and that subsequent embryogeny is characteristic of the other sex. An organ which is determined before the turning point is reached will be like that of the former sex; one which is determined after the turning point will be like that of the latter sex. An individual produced as a result of the change postulated above is a time-mosaic of male parts and female parts whose embryonic segregations occur at different times in development. This concept of development has also been applied to intermediates in general and has been termed the time-of-determination theory (Shull, 1930). Since intermediates may conceivably be produced without any time intervals between the determination of their different structures, it is desirable to discover whether such a succession of events does occur.

Intermediates of such a nature that they may be used to test the applicability of the time-of-determination theory are not common. But when in a species of organism there exist two forms of individuals built on the same general structural plan, but differing with respect to recognizably homologous parts, definite information regarding the time of determination of the differential parts can be gained, provided each of these forms can be converted by stages into the other. Previous to Shull's (1930) work on aphids, efforts to obtain this information have dealt almost exclusively with intersexes which are the product of bisexual reproduction. The genetic composition of such intersexes is presumably variable. Intermediate aphids parthenogenetically produced have the advantage of allowing only a minimum of opportunity for the

¹ Contribution from the Department of Zoölogy, University of Michigan.



embryonic process to be influenced by genetical variation, and therefore insuring that genetic and developmental factors will not be confused in the results.

The test of serial embryonic segregation (embryonic determination) lies in obtaining intermediates by an approach from opposite extremes. Shull made such a test on aphids which could be changed from parthenogenetic to gametic, and from gametic to parthenogenetic. This change from one form to the other results in the production of many aphids which are structurally intermediate between the gametic and parthenogenetic. If the time-of-determination theory be applicable to the intermediates produced, the order in which the structures change should be the same in both; but, since in one of the changes a given organ should appear, while in the opposite change that organ should be lost, the combination of characters in the intermediates produced should be quite different in the two cases. In fact, they should be complementary in the complex of organs concerned.

In Shull's early experiments the combination of characters in the aphids obtained by this method were not complementary; the order of the change was apparently reversed, while the development of the intermediate characters was identical regardless of the direction of change. To explain these results which appeared to oppose the time-of-determination theory, Shull discusses the possibility of a physiological level hypothesis as an alternative. According to the physiological level theory, the degree of intermediacy might depend upon the state in which some physiological property existed at some critical time. If all the differentiating structures responded equally to changes in the level of this physiological process, the question of the order of determination would not arise. All organs would appear to be segregated at the same time, or during the same part of the embryogeny. Since, however, one structure might respond to a small increase of a hormone, for example, so that it became changed, while another less susceptible does not, there develops a situation in which a difference in the time of determination of these structures may be suspected, although such a difference were non-existent. This explanation is opposed to the theory that the time of embryonic segregation determines the combination of characters in the intermediate individuals. Shull's (1933) final study of a heterogeneous group of intermediate aphids of the gametic-parthenogenetic types showed that about three-fourths fell into categories to which the time-of-determination theory was applicable, and nearly one-fourth showed irregularities which could not be made to harmonize with this theory, or which required shifts in the order of determination of the several organs studied.

Shull (1937) reports a different type of intermediacy, one in which the intermediates are structurally between the winged and wingless parthenogenetic forms. The intermediates reported in the present paper are of the winged-wingless type. In fact, the intermediates discussed here were a part of the 9,152 reported by Shull (1937).

Shull (1937), after discussing many different possible hypotheses which might account for intermediate-winged aphids, states that whether intermediates are in any way dependent on the *direction* of some physiological change could not be judged from the experimental methods of producing them. Then he adds that analysis of the characters of intermediates will be needed to answer this question. This investigation represents an effort to make a more thorough study than one involving only external characters to which Shull (1937) confined his studies. There are many limitations to this method, for the possible internal development of an organ before there is any external evidence may be of great importance in explaining the developmental basis for intermediate-winged aphids.

The structures involved in intermediate-winged aphids are more minute than in the gamic-parthenogenetic intermediates (Shull, 1930) which were examined internally by gross dissection. Accordingly, the winged-wingless intermediate aphids of this investigation, after their external organs were rated, were prepared for histological and cytological studies of the internal structures, thus making possible a thorough analysis of the whole intermediate complex.

The analysis of the intermediates so studied provides the subject matter of this paper, which records attempts to test not only the relative merits of the time-of-determination and physiological level theories as applied to intermediate winged aphids, but to determine the order of embryonic segregation if the time-of-determination theory proved applicable; and to consider other related developmental phenomena. The author wishes to express here his great indebtedness to Professor A. Franklin Shull under whose direction this work was done.

THE STOCKS OF INTERMEDIATES

All aphids used in this study were from stocks which Professor A. Franklin Shull had been keeping for several years under laboratory conditions. They were from two different strains of the species *Macrosiphum solanifolii*, which have been designated the 1923 and 1931 (Shull, 1937) stocks. Shull (1937) describes in detail the experimental methods used in the production of winged-wingless parthenogenetic female aphids.

EXTERNAL AND INTERNAL DIFFERENTIATING CHARACTERS

A complete description of the external characters of typical winged and wingless forms of *Macrosiphum solanifolii* has been given by Patch (1915). The winged female differs from the wingless in possessing wings, three ocelli on the head, wing muscles in the thorax, extra sensoria on the third segment of the antennæ (4-6 in the wingless, 15-18 in the winged), and dark color in the third segment of the antennæ. The first three organs named proved of the most critical value for this study.

Internal differences between the winged and wingless are, for the most part, correlated in some way with the external distinguishing characters. The wing muscles of the winged individuals are striated muscles, both vertical and horizontal in position. The protocerebral lobes of the brain to which the ocellar nerves are connected in the winged type show greater development than in the wingless forms. No qualitative or quantitative differences in the "symbiotic organ" were observed. There is a practical difficulty involved in making quantitative comparisons as the number of "symbionts" gradually decreases with the age of an aphid (Uichanco, 1924).

TECHNIQUE

Toto mounts of intermediate-winged aphids were of some value in comparing the relative development of the median ocellus to the lateral ocelli. Of a variety of methods used for this toto mount work, the best proved to be fixation in Bouin's fluid, dehydration with ethyl alcohol, clearing with xylol and mounting in dammar.

Histological and cytological studies which involve quantitative considerations necessitate a technique which results in almost faultless preparations. If the tissue is to be satisfactory there must be no tearing, shattering or distortion. A great many techniques with many variations were tried in an effort to obtain the most satisfactory method. None of the techniques proved satisfactory until the n-butyl method for animal tissues described by Stiles (1934) was used for dehydration and infiltration. Legs, wings, and antennæ were snipped off with DeWecker eye scissors to facilitate handling and to improve sectioning of the aphids. The sections were cut 7 micra and stained with Ehrlich's acid hematoxylin as a nuclear stain and erythrosin for the cytoplasm.

DESCRIPTION OF INTERMEDIATES

General Anatomy

A diagnosis of intermediacy of the winged-wingless aphids has generally been based on external features and as a result intermediates of

this type have been defined as aphids that have started to develop wings but have failed to complete such development. That this is not by itself a diagnostic feature of intermediacy is shown by the fact that there is histological evidence of internal intermediacy without external wings. Nevertheless, the investigation described in this paper is limited almost exclusively to intermediates which could be recognized by their wing characters, because of the enormous amount of labor involved in making histological preparations to identify intermediacy from the internal organs.

The wing character in intermediates is very variable. The partially developed wings in an intermediate may vary in development from a barely perceptible roughness in the thoracic region to .95 of normal development, the latter type being very unusual. Drooping wings was a character common to all the seven species of winged intermediates described by Baker and Turner (1916), and they considered this due to a lack of supporting muscles. While many wings examined by the writer were badly crumpled and misshapen, there was no decided tendency to droop; and even if there had been, it is doubtful if it could have been explained on the basis of a lack of muscle development as many intermediates have muscles well developed in proportion to the amount of wing. Unusual cases were those in which the wings were about one-half developed and would have shown muscle through the cuticula of the dorsal thorax but did not because of a degeneration (to be discussed later) which destroyed the muscle. Fat globules could be seen through the cuticula where muscles would normally have been seen.

Antennal color may be like that of the winged or wingless, or it may be some grade between these extremes (Tables I to IX). The sensoria, although variable, are often no greater in number than in the wingless,

EXPLANATION OF PLATE I

FIG. 1. Photomicrograph of a transverse section of the head of aphid 1103 d (4), showing on the left side considerable development internally of the ocellus before there is any thickening of the cuticula to form a lens ($\times 134$).

FIG. 2. Photomicrograph of a transverse section of the mesothorax of P W 48 (1), showing the wing muscle of a fully-winged aphid ($\times 105$).

FIG. 3. Photomicrograph of a transverse section of the mesothorax of aphid 1087 g (2), showing the reduced wing muscle of an intermediate ($\times 117$).

FIG. 4. Photomicrograph of a transverse section of the mesothorax of P 41 (1), a fourth instar winged aphid ($\times 88$).

FIG. 5. Photomicrograph of a transverse section of the mesothorax of P 25 (2), a fourth instar intermediate aphid showing degenerating wing muscle ($\times 95$).

FIG. 6. A photomicrograph taken with oil immersion lenses to show the detail of degenerating wing muscle of a fourth instar aphid, P 25 (3). The light-colored tissue in the upper part of the picture is normal muscle. Note especially the dark spherical degenerating cells between the muscle masses ($\times 917$).

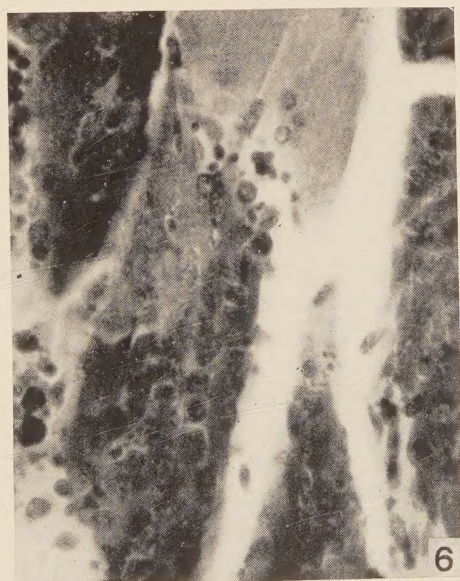
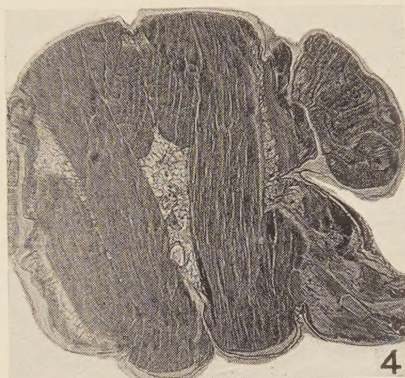
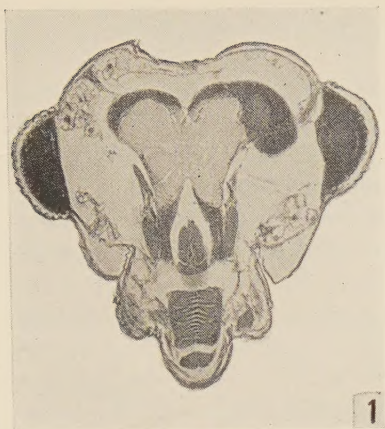


PLATE I

and uncommonly approach the high degree of development in the winged (Tables I to IX).

Rudimentary ocelli are frequently present in various degrees of formation. There are many cases in which no ocelli can be seen externally with either a dissection or compound microscope, but histological preparations gave positive evidence of a partly formed structure internally (Fig. 1).

The histological structure of the wing muscle in intermediate aphids may be characteristic of that in the typical winged or it may be degenerate in varying degrees. Wing muscles (Fig. 2) which in the typical aphid occupy so much of the space in the thorax, in the intermediate (Fig. 3) are usually less extensive. Degeneration of larval muscles resulting from histolysis in insects with a complete metamorphosis is well known. But in groups with a paurometabolous (incomplete) metamorphosis there is usually a continuous transformation of the internal organs from the immature stages to the adult without histolysis of muscle or other internal structures. With these well-established facts in mind, one would not expect to find degenerating wing muscles in intermediate aphids unless it developed that they possessed marked physiological differences from normal types. However, nearly one-half of the intermediates studied showed wing muscle with some degree of degeneration. This degeneration expresses itself in conditions which range from almost complete dissolution of the wing muscle to muscle that can be told from normal only with difficulty. The histology of this degenerating wing muscle is in some respects unlike that which has been described for the histolysis of the wing muscle of the queen ant, *Lasius niger*, by Janet (1907). The degeneration in the ant wing muscle takes place soon after the nuptial flight. At the beginning of histolysis, striations may be seen in the muscle but they become faint and finally disappear. Then the sarcostyles undergo disintegration but the nuclei persist for some time. The story of histolysis in wing muscles of the intermediate-winged aphid differs most from that of the ant in that embryonic muscles are involved.

The earliest stages in which the histolysis has been observed in the thoracic muscle of intermediate-winged aphids is the fourth instar. At this stage in the normal metamorphosis the wing muscle has about completed its development and the muscle generally exhibits the character of that of the imago.

Comparison of a fourth instar with normal muscle (Fig. 4) and a fourth instar which shows degenerating muscle (Fig. 5) will indicate some of the general differences to be found. The intermediate nature of the muscle in Fig. 5 is indicated by the small amount. Both aphids

were prepared by a similar technic and stained with Ehrlich's hematoxylin. The degenerate muscle in Fig. 5 is distinctly basophilic whereas the normal muscle in Fig. 4 is decidedly eosinophilic. This basophilic nature of muscle is characteristic of the periphery of the developing muscle mass of the earlier instars. This might suggest embryonic rather than degenerating muscle, but in normal developing muscle, while the periphery of a muscle where mitoses are taking place most rapidly may be basophilic, the median portion which has earlier become differentiated is eosinophilic. So in embryonic muscle the differentiation is from the center of the muscle mass to the periphery. There are, however, instances in which the degeneration of muscle is slight and these simulate somewhat the developing wing muscle of early instars.

Peripheral degeneration is interpreted as a result of the greater susceptibility to environmental influences which embryonic cells may show. Mottram (1913) with radiation has shown that *Ascaris* eggs are eight times as susceptible during division as during the resting stage. That is, there are eight times as many deaths following an exposure made during mitosis as during the later period. It has also been observed that cancer tissue is much more susceptible to radiation when it is growing rapidly than when it is nearly stationary. The last part of the aphid wing muscle to remain embryonic is the periphery and if degeneration of wing muscles, as the author believes, is due to an environmental influence, the peripheral tissue would be most affected. Figure 6 is a high power photomicrograph of the wing muscle in a fourth instar intermediate-winged aphid showing something of the more detailed structure of unusually degenerate muscles. The very dark spherical cells, lacking uniformity in size, which are to be seen in the spaces between the masses of muscles, are in advanced stages of cell degeneration. These cells in the advanced stages of degeneration seen in intramuscular spaces were doubtless at the periphery of the muscles shown and were least developed when the degenerating influence became effective. It may well be that they were more susceptible because of their extremely embryonic character. As is seen, these cells stain intensely with hematoxylin. The small amount of light colored muscle in the upper part of the picture is normal tissue which had reached the definitive stage and was unaffected by whatever influence caused the embryonic cells to degenerate. It was noted that not only mature wing muscles, but other definitive muscles of the body do not seem to suffer degeneration even though there may be marked histolysis of the embryonic muscles present.

Figure 6, in which histolysis is most active, is favorable material for studying some of the details of the process of degeneration. A loose-

ness of the muscle tissue is apparent, distinct outlines of muscle cells may be seen which normally would be so closely crowded together that they could not be delimited from one another. In a given mass of degenerating muscle the tissue least affected is that farthest from the periphery.

Adult intermediate-winged aphids do not often show as much degeneration of wing muscles as is illustrated by Fig. 7. The wing muscles in Fig. 7 have suffered degeneration to the point where only unstriated loosely fibrous strands of tissue crowded with irregular, densely staining pyknotic nuclei remain. Bardeen (1900) points out that nuclei with a strong affinity for stain are characteristic of degenerating muscle



FIGS. 7-8

FIG. 7. Photomicrograph of a transverse section of the mesothorax of aphid 220 e, an intermediate with .6 wing development. The vertical wing muscles are loose, fibrous, unstriated strands of tissue, an example of extreme muscle degeneration in the adult ($\times 115$).

FIG. 8. Photomicrograph of a transverse section of the mesothorax of aphid 1063 g (2), with .22 wing development and histologically normal wing muscle ($\times 128$).

in the pig embryo. Associated with the degenerate vertical muscle on the right side may be seen a group of either degenerate muscle cells or phagocytes distended with muscle debris. Some of the larger of these spherical bodies are muscle cells undergoing dissolution, but some of the smaller have the characteristics of leucocytes which are filled with digesting muscle fragments.

The longitudinal as well as the vertical muscles are involved in the degeneration process. The longitudinal muscles have so completely degenerated in Fig. 7 that there are only a few degenerate fragments left. The question whether a wing muscle degenerates or not depends,

as has been pointed out, chiefly on its stage of development when conditions for degeneration obtain. The importance of wing muscle degeneration in intermediate-winged aphids in its relation to the time of embryonic determination of organs will be considered in another part of this paper.

It would be logical to examine the developing wings and ocelli in nymphal winged intermediate aphids with degenerating muscles to see if they experience any histolysis. If reference is again made to Fig. 5, the wing will be found to be reduced in size for a fourth instar and there are indications of degeneration. Cells of hypodermis of the lower and upper side of the wing, instead of being elongated as in the normal developing wing, tend to become spherical in some instances, and show a marked affinity for hematoxylin as do the degenerating muscle cells. Other disintegrating cells do not become spherical, but the cytoplasmic part of the cell can plainly be seen to be breaking down. The wing tissue in process of degeneration exhibits a marked affinity for hematoxylin. Degenerate cells are scattered in the space between the upper and lower sides of the wing bud.

From this study of the internal microscopic anatomy of intermediate aphids it is seen that there are two distinct anatomical situations, one in which the organs concerned with intermediacy are normal in histology, the other in which at least the wing muscle and developing wing bud show more or less degeneration.

Measures of Intermediacy

If intermediate aphids owe their intermediacy to a change in embryonic development from that characteristic of one normal type to that of the other type (winged to wingless or wingless to winged), and if the differentiating structures are determined at different times, it must be expected that these structures would attain different grades of advancement in different individuals. These grades of advancement are, indeed, the criteria by which the time-of-determination theory must be judged. It is necessary, therefore, to have some means of recording their degrees of development. Objective methods of measuring structures were employed when practicable. To the other characters numerical values were arbitrarily given, 0 representing the extreme found in one form, and 4 the opposite extreme in the other type. This classification of the external characters was done on living animals which had been etherized. A binocular dissection microscope usually gave magnification enough for external examination, although there were times when a question arose concerning the presence of an ocellus pri-

mordium, and this would be decided with the aid of a compound microscope.

With respect to ocelli, 0 designates a complete absence of ocelli, which is typical for the wingless viviparous females; 4, the fully developed ocelli of a winged individual; and values between these two extremes represent varying degrees of intermediacy. For example, 1 would mean ocelli one-fourth developed.

The light color of the antennae in the wingless female was rated 0, and the dark color of the winged as 4, with intergrades between these two numbers of 1, 2, and 3. An actual count of sensoria on each antenna was made; and while there was no effort to distinguish between right and left, each was tabulated separately.

Wings were used in this study as a standard of intermediacy for rough classification; that is, reference to degrees of intermediacy without regard to internal structures was based on the amount of wing development. When an aphid is said to possess a low degree of intermediacy, it means that there is little wing development although the wing muscles might be well developed; and conversely, when a high degree of intermediacy is designated, it means that the wing development is relatively great irrespective of other structures concerned in intermediacy. The wing was chosen as a standard of reference because it is an external differential character which can most easily be rated. Wings were rated from 1 to .01, the former number meaning that an aphid was fully winged, and the latter that the wing was .01 of the normal length. Some wings were less than .01 of the length of normal wings; in fact, there was a fair-sized group in which the wings were represented only by a roughness of the thoracic region where wing buds should appear. These in the raw data were termed subintermediates, to indicate a very low degree of intermediacy. These subintermediates in Tables I to IX, are indicated by a negative sign (—) after .01, as .01 —.

The degree of internal intermediacy was judged by quantitative means. For this work transverse serial sections were found most suitable. Measurements of all the internal differential organs were taken with an ocular micrometer. In rating the ocelli, the diameter was first measured and then the number of sections counted.

The wing musculature in transverse sections (Fig. 2) consists of two inner and two outer vertical muscles, together with what appears to be a single rectangular mass of longitudinal muscles divided by a median fissure. Only the maximum width of the inner and outer vertical muscles was measured, but the total area of the longitudinal muscles was computed from measurements of both width and height. A record of the total number of sections containing vertical wing muscles was

made. The final quantitative rating of the wing muscle development was based largely on the width of the vertical muscles, for the correlation between width of muscle and number of sections was close enough to make the latter measurement of value largely as a check on the former. A careful comparison of the longitudinal and vertical muscles revealed that the longitudinal, proportionately, were consistently less developed than the vertical in intermediate forms. Because the correlation between the two sets of muscles proved to be rather constant, little was to be gained by using the longitudinal muscles as a quantitative measure of intermediacy. That there might be a close correlation between these two groups of muscles seemed probable from a study of their development in the early embryogeny of the aphid, for it was learned that both appear at about the same time. At least, when the vertical muscles were found the longitudinal were always present, and both showed about the same relative development. This made it doubtful whether there was a difference in the time of determination for these two different sets of muscles, although there could be.

The differential characters which proved of greatest worth as measures of intermediacy in this investigation were the wings, ocelli, and wing muscles, and consequently these were most carefully rated. After the raw data on these structures had been obtained they were compared with the mean for the same organs in what were considered typical winged individuals. In tables of these values the development of these differentiating structures is expressed in percentages.

By means of the differentiating characters described above, a diagnosis of the intermediates of this study is made with the results presented in Tables I to IX. The data are grouped according to conclusions to be drawn, but for the present this may be ignored. The reasons for so arranging the data that conclusions are anticipated is that it saves repeating this material in a general table.

Expected Composition of Intermediates Under Various Suppositions as to Order of Determination and Direction of Change

As seen above, Goldschmidt has applied the time-of-determination theory to intersexes. In accordance with that theory, organs which are the first to be changed in successive offspring must be the latest to be determined in development, while the latest to change in successive offspring are the earliest to be determined. The order of embryonic segregation is the reverse of the order of modification in successive offspring. If this theory is applicable to intermediate aphids produced

during a transition from winged to wingless or wingless to winged, then it should be possible to determine the order of segregation if the direction of change is known.

Whether the time-of-determination theory is correct in a given instance cannot be proven by finding that all characters of the two types of individuals change simultaneously. In fact, such a circumstance would make possible the supposition that there was no difference in the time of determination of the different organs. But even though several structures did appear at the same time in the embryo, the possibility remains that they may not all have been determined at the same moment, as there could be a difference in the intervals between the time of physiological determination of two or more organs and their morphological appearance, and that difference might make possible their simultaneous appearance in development even though their determinations coincided in time. Nor is the time-of-determination theory confirmed by the discovery that certain characters change in earlier offspring than do others. If intermediates that are near to the one extreme type always possess a given set of characters, and those near the other extreme type always have another set of characters, these facts furnish no support to the time-of-determination hypothesis. What is needed to prove the theory is to demonstrate (1) that when the intermediate condition is approached from one of the extremes, the changes in successive offspring shall take place in a definite order, and (2) that when the intermediate condition is approached from the opposite extreme the same order of change shall occur.

Whether the conditions necessary for confirmation of the time-of-determination theory are met in a given case of intermediacy can be tested by (1) considering all the possible types of individuals that would result from all conceivable orders of determination of the several differentiating structures concerned in intermediacy, and (2) tabulating the data on the quantitative development of each differentiating structure in all the intermediates obtained. Then test which assumption each intermediate best fits. If more intermediates fit one assumption than others, that assumption is favored. With three different organs (wings, ocelli, and wing muscles) to be considered there are six different possible orders of embryonic determination which are shown below in the diagram as numbered assumptions. The order of determination is indicated by the arrows, that is, in I the wings are determined first, then wing muscle, and the ocelli last.

Six different possible orders of determination for three differentiating organs

Assumption I.	Wings	→	Wing Muscle	→	Ocelli
Assumption II.	Ocelli	→	Wing Muscle	→	Wings
Assumption III.	Wing Muscle	→	Ocelli	→	Wings
Assumption IV.	Wings	→	Ocelli	→	Wing Muscle
Assumption V.	Ocelli	→	Wings	→	Wing Muscle
Assumption VI.	Wing Muscle	→	Wings	→	Ocelli

For a given order of determination there will be a number of intergrades as illustrated below in Assumptions IV A and IV B. The "A" part of Assumption IV shows the various kinds of intermediate-winged aphids which would be produced as a result of a change from winged to wingless forms, with the order of determination wings, ocelli and wing muscle. Assumption IV B indicates the different kinds of intermediate-winged aphids produced with the same order of determination as in Assumption IV A, but during a transition from wingless to winged types. The third intermediate from the top of Assumption IV A would deviate from a normal winged individual by possessing normal wings, slightly reduced ocelli and much reduced wing muscle. If a detailed chart of each of the six assumptions is made out and then an attempt is made to determine which of the six possibilities for embryonic determination each intermediate best fits, it is obvious that each intermediate form will fit equally well two of the possibilities, depending on the order of determination, as will be illustrated later. If more intermediates fit one assumption than others, that assumption is favored. The above procedure was followed with the intermediate-winged aphids of this investigation. Three structures, wings, ocelli and wing muscles, showed enough intergradations in development to make them satisfactory for such a study. As previously stated, an effort was made to discover to which one of the six different possible orders of embryonic determination the intermediate-winged aphids of this research tabulated in Tables I to IX best conformed.

It will be seen that for each possible order of embryonic segregation there are two ways in which the characters may be combined, depending on the direction of change. The diagrams below illustrate the different combinations of intermediate characters as they occur in different winged-wingless aphids for selected assumptions. Each diagram shows a complete transition from typical winged to wingless forms or vice versa. Read from left to right to determine the nature of any given intermediate.

If the transformation is from winged to wingless with the order of determination wings, ocelli and wing muscle, Assumption IV A, the

during a transition from winged to wingless or wingless to winged, then it should be possible to determine the order of segregation if the direction of change is known.

Whether the time-of-determination theory is correct in a given instance cannot be proven by finding that all characters of the two types of individuals change simultaneously. In fact, such a circumstance would make possible the supposition that there was no difference in the time of determination of the different organs. But even though several structures did appear at the same time in the embryo, the possibility remains that they may not all have been determined at the same moment, as there could be a difference in the intervals between the time of physiological determination of two or more organs and their morphological appearance, and that difference might make possible their simultaneous appearance in development even though their determinations coincided in time. Nor is the time-of-determination theory confirmed by the discovery that certain characters change in earlier offspring than do others. If intermediates that are near to the one extreme type always possess a given set of characters, and those near the other extreme type always have another set of characters, these facts furnish no support to the time-of-determination hypothesis. What is needed to prove the theory is to demonstrate (1) that when the intermediate condition is approached from one of the extremes, the changes in successive offspring shall take place in a definite order, and (2) that when the intermediate condition is approached from the opposite extreme the same order of change shall occur.

Whether the conditions necessary for confirmation of the time-of-determination theory are met in a given case of intermediacy can be tested by (1) considering all the possible types of individuals that would result from all conceivable orders of determination of the several differentiating structures concerned in intermediacy, and (2) tabulating the data on the quantitative development of each differentiating structure in all the intermediates obtained. Then test which assumption each intermediate best fits. If more intermediates fit one assumption than others, that assumption is favored. With three different organs (wings, ocelli, and wing muscles) to be considered there are six different possible orders of embryonic determination which are shown below in the diagram as numbered assumptions. The order of determination is indicated by the arrows, that is, in I the wings are determined first, then wing muscle, and the ocelli last.

Six different possible orders of determination for three differentiating organs

Assumption I.	Wings	→	Wing Muscle	→	Ocelli
Assumption II.	Ocelli	→	Wing Muscle	→	Wings
Assumption III.	Wing Muscle	→	Ocelli	→	Wings
Assumption IV.	Wings	→	Ocelli	→	Wing Muscle
Assumption V.	Ocelli	→	Wings	→	Wing Muscle
Assumption VI.	Wing Muscle	→	Wings	→	Ocelli

For a given order of determination there will be a number of intergrades as illustrated below in Assumptions IV A and IV B. The "A" part of Assumption IV shows the various kinds of intermediate-winged aphids which would be produced as a result of a change from winged to wingless forms, with the order of determination wings, ocelli and wing muscle. Assumption IV B indicates the different kinds of intermediate-winged aphids produced with the same order of determination as in Assumption IV A, but during a transition from wingless to winged types. The third intermediate from the top of Assumption IV A would deviate from a normal winged individual by possessing normal wings, slightly reduced ocelli and much reduced wing muscle. If a detailed chart of each of the six assumptions is made out and then an attempt is made to determine which of the six possibilities for embryonic determination each intermediate best fits, it is obvious that each intermediate form will fit equally well two of the possibilities, depending on the order of determination, as will be illustrated later. If more intermediates fit one assumption than others, that assumption is favored. The above procedure was followed with the intermediate-winged aphids of this investigation. Three structures, wings, ocelli and wing muscles, showed enough intergradations in development to make them satisfactory for such a study. As previously stated, an effort was made to discover to which one of the six different possible orders of embryonic determination the intermediate-winged aphids of this research tabulated in Tables I to IX best conformed.

It will be seen that for each possible order of embryonic segregation there are two ways in which the characters may be combined, depending on the direction of change. The diagrams below illustrate the different combinations of intermediate characters as they occur in different winged-wingless aphids for selected assumptions. Each diagram shows a complete transition from typical winged to wingless forms or vice versa. Read from left to right to determine the nature of any given intermediate.

If the transformation is from winged to wingless with the order of determination wings, ocelli and wing muscle, Assumption IV A, the

wings would be most developed, the ocelli less, and the wing muscles least. If, however, the transition is from wingless to winged with the order of determination the same as in Assumption IV, then the wings will be least developed, the ocelli more, and the wing muscle most as shown in Assumption IV B. Thus it is possible to have two graduated series, one showing intermediates resulting from a change from winged

ASSUMPTION IIIA REGARDING TIME OF DETERMINATION

Direction of change—winged to wingless

Combinations of intermediate characters		
<i>Wing Muscle</i>	→ <i>Ocelli</i>	→ <i>Wings</i>
Normal	Normal	Normal
Normal	Normal	Slightly reduced
Normal	Slightly reduced	Much reduced
Slightly reduced	Much reduced	Very much reduced
Much reduced	Very much reduced	None
Very much reduced	None	None
None	None	None

ASSUMPTION IIIB REGARDING TIME OF DETERMINATION

Direction of change—wingless to winged

Combinations of intermediate characters		
<i>Wing Muscle</i>	→ <i>Ocelli</i>	→ <i>Wings</i>
None	None	None
None	None	Slightly developed
None	Slightly developed	More developed
Slightly developed	More developed	Highly developed
More developed	Highly developed	Normal
Highly developed	Normal	Normal
Normal	Normal	Normal

ASSUMPTION IVA REGARDING TIME OF DETERMINATION

Direction of change—winged to wingless

Combinations of intermediate characters		
<i>Wings</i>	→ <i>Ocelli</i>	→ <i>Wing Muscle</i>
Normal	Normal	Normal
Normal	Normal	Slightly reduced
Normal	Slightly reduced	Much reduced
Slightly reduced	Much reduced	Very much reduced
Much reduced	Very much reduced	None
Very much reduced	None	None
None	None	None

ASSUMPTION IVB REGARDING TIME OF DETERMINATION

Direction of change—wingless to winged

Combinations of intermediate characters		
<i>Wings</i>	→ <i>Ocelli</i>	→ <i>Wing Muscle</i>
None	None	None
None	None	Slightly developed
None	Slightly developed	More developed
Slightly developed	More developed	Highly developed
More developed	Highly developed	Normal
Highly developed	Normal	Normal
Normal	Normal	Normal

to wingless, and another including totally different kinds of intermediates when the change is from wingless to winged. The aphids of these two series, Assumptions IVA and IVB, are complementary in their composition; that is, in one the wings are large, in the other small, and the same relationship holds for the other structures. Figures 7 and 8 picture this complementary condition in the wing muscles; Fig. 7 is an intermediate with wings .60, and degenerate wing muscle .03 developed, while Fig. 8 is rated with wings .22 and non-degenerate wing muscle .66.

As indicated above, any intermediate aphid should fit equally well two of the six assumptions for different orders of determination because an intermediate of a structural composition that should result from an assumed order of determination, such as wing muscle, ocelli, wings, Assumption III B, could also result from the reverse order of determination, wings, ocelli, wing muscle, Assumption IV A, if the direction of change were reversed. The above-mentioned assumptions illustrate this, for the composition of an aphid which fits Assumption III B is identical with one which fits Assumption IV A. In other words, an intermediate produced as a result of a transition from winged to wingless in Assumption IV A is the same in composition as one resulting from a conversion from wingless to winged in Assumption III B. Hence, all that is shown by such a classification and testing of data would be that the order of determination was one of two possibilities, one the reverse of the other, and unless the direction of change is known the elimination of one of these possibilities is out of the question.

It was not known from the conditions under which these intermediate-winged aphids were reared whether a given intermediate was produced as a result of change from winged to wingless or the reverse. Unlike the gamic-parthenogenetic intermediates found by Shull, there was no gradual change from one type to the other. Shull found it possible to get stocks producing mostly gamic females and then effect

a gradual change to parthenogenetic types with many intermediates appearing during the change. Presumably the intermediates produced under these conditions were the result of a gamic-to-parthenogenetic change. In the experiments in which intermediate-winged forms appeared there were usually both winged and wingless aphids with a few intermediates. Several females were placed on one plant and one aphid might be giving birth to offspring which were intermediate as the result of change in one direction while another could be producing intermediates as a result of change in the opposite direction. And even if a female were to be reared in isolation on a plant, little could probably be told about the direction of change which resulted in intermediacy for the winged and wingless offspring occur in a very erratic manner through the family. It is also conceivable that in a given experiment an aphid could produce intermediate offspring as the result of a change in one direction at one time, and other intermediates from the opposite direction of change at another time. If this occurred there would be no way by which the direction of change could be detected from the daughters. Shull (1928, 1929) has shown that light and temperature modify the development of wings but these agents cannot be relied on to change the course of development in all individuals in the strains now available which are producing the intermediates. Thus it will be seen that the experiments themselves afford no reliable method whereby the direction of change can be ascertained.

However, this investigation has revealed what is considered to be a means of deciding the direction of change which results in intermediacy. As has been stated above, there were found to be two different classes of intermediates based on internal structures, namely, (1) those with histologically normal wing muscles and (2) those with degenerative wing muscles. It is postulated by the author that degenerate muscles are the consequence of a transformation from winged to wingless forms. Development is assumed to have begun with factors favoring wing production dominant, then a physiological change taking place which not only inhibited further development of characters associated with wings, but brought about more or less degeneration of the wing muscle already produced. When a change takes place in the opposite direction, that is, a conversion from wingless to winged, there is no degeneration of the organs of the winged type because in this transition the physiology changes from one in which those organs are not present to one which favors their development. With conditions favorable for the production of the structures characteristic of winged females, these organs undergo normal histological development. The facts are that about 56 per cent of the intermediates of this investigation were characterized

by normal muscles, while about 44 per cent possessed degenerate muscles. This interpretation of the two classes of intermediates based on the nature of wing muscle affords a means of determining the direction of change in a given individual so that it is possible to classify each as the result of a conversion from winged to wingless or wingless to winged. In Tables I to IX inclusive, all aphids listed under the "A" assumptions possess degenerate wing muscles; all those listed under the "B" assumptions are wingless-to-winged transformations.

By a study of these Tables (I to IX inclusive) the conclusion that normal and degenerate wing muscles are correlated with some direction of change is deduced from the way in which the tabulated material fits the various assumptions. If degeneration of muscles were a purely fortuitous phenomenon, with one aphid as likely as another to be affected regardless of the direction of transformation producing intermediacy, every Assumption "A," of which there are six, should include some intermediates, which according to the conditions of their classification would possess degenerate muscles. This expectation is not realized. Moreover, Assumptions III and IV are complementary as discussed above, and about 50 per cent of the aphids of this investigation conform to Assumption IV B; yet there is not an aphid to be found conforming to its complementary Assumption III A. If degeneration of wing muscle were determined by mere chance, it would seem very probable that some of the aphids classified in Assumption IV B would have come under the influence causing muscle degeneration, thus changing their classification to Assumption III A. The converse of this statement is equally true; if the wing muscle in intermediates postulated to be changing from wingless to winged were decided only by chance, there should also be aphids which would fit Assumption III B; but none were found. There seems no other way to explain why aphids with the intermediate characters of Assumption IV B, normal muscle, should not have possessed degenerate muscles which would fit them to Assumption III A except that normal muscle is associated with a certain direction of change which is here postulated to be from wingless to winged. Thus it will be seen that the histological character of intermediate-winged aphids is correlated with direction of change. Whether it means a winged-to-wingless or wingless-to-winged transition depends on the correctness of the theory that degenerating muscles result from a change in physiology to which the immature wing muscles are susceptible.

This method of establishing the direction of change which results in intermediacy makes it possible to eliminate one or the other of Assumptions III and IV which are complementary as far as the development

TABLES I-IX

Tables I, II, III, IV, V, VI, VII, VIII and IX contain the data regarding the development of the differentiating organs of intermediacy of winged-wingless aphids. The data are arranged, in so far as possible, to fit the various assumptions made.

TABLE I

Composition of intermediate aphid fitting Assumption I A. Order of determination: wings→wing muscle→ocelli. Change from winged to wingless. Degenerate wing muscle.

Slide No.	Characters to which Assumption I A is fitted			Number of sensoria	Antennal color
	Wing length	Wing muscle development	Ocellar development		
1196 c (7)	.50	.49	.41	5-6	0

TABLE II

Composition of intermediate aphid fitting Assumption I B. Order of determination: wings→wing muscle→ocelli. Change from wingless to winged. Normal wing muscle.

Slide No.	Characters to which Assumption I B is fitted			Number of sensoria	Antennal color
	Wing length	Wing muscle development	Ocellar development		
1170 c (2)	.03	.28	.35	5-4	0

TABLE III

Composition of intermediate aphids fitting Assumption II A. Order of determination: ocelli→wing muscle→wings. Change from winged to wingless. Degenerate wing muscle.

Slide No.	Characters to which Assumption II A is fitted			Number of sensoria	Antennal color
	Ocellar development	Wing muscle development	Wing length		
1083 (1)	.35	.23	.15	4-8	0
226 e	.35	.16	.10	6-7	0
245 f	.23	.20	.10	6-8	0
288 e (4)	.15	.12	.08	5-5	0
288 e (5)	.11	.06	.05	5-6	0
909 a	.23	.08	.04	6-5	0
40 c (2)	.22	.20	.02	7-7	0
905 b (4)	.35	.16	.02	10-5	1
905 b (5)	.35	.33	.02	10-5	1
1016 a	.35	.20	.01	4-4	0
1087 g (4)	.17	.16	.01	5-5	0

TABLE IV

Composition of intermediate aphid fitting Assumption II B. Order of determination: ocelli→wing muscle→wings. Change from wingless to winged. Normal wing muscle.

Slide No.	Characters to which Assumption II B is fitted			Number of sensoria	Antennal color
	Ocellar development	Wing muscle development	Wing length		
490 g (1).....	.66	.91	.95		3

TABLE V

Composition of intermediate aphids fitting Assumption IV A. Order of determination: wings→ocelli→wing muscle. Change from winged to wingless. Degenerate wing muscle.

Slide No.	Characters to which Assumption IV A is fitted			Number of sensoria	Antennal color
	Wing length	Ocellar development	Wing muscle development		
P-27.....	1.00	.95	.90		
285 e (2).....	.80	.59	.12	5-6	0
285 e (1).....	.80	No sections of head	.23	6-6	0
286 f.....	.80		.08	7-7	1
282 e.....	.75		.49	8-8	2
288 e (1).....	.75	.47	.12	5-6	0
1196 c (2).....	.70	.47	.28	5-7	0
428 d (2).....	.70	.59	.45		
235 b.....	.65	.59	.57	13-13	3
296 b (3).....	.60	.46	.08	7-7	0
285 c (2).....	.60	.46	.28	7-8	1
279 e.....	.60	.46	.06	7-7	1
220 e.....	.60	.46	.03	6-5	0
1085 g (1).....	.60	.46	.41	6-?	1
1196 c (11).....	.60	.41	.23	6-6	0
488 d (2).....	.50	.46	.41		0
220 d.....	.50	.35	.01-	6-6	0
1021 a.....	.50	.46	.25	5-4	0
1188 b.....	.50	.46	.16	9-6	0
282 c (1).....	.40	.33	.08	7-7	1
212 b.....	.40	.33	.08	5-6	0
1130 f.....	.40	.33	.16	5-6	0
280 c (2).....	.35	.23	.01-	7-8	2

TABLE VI

Composition of intermediate aphids fitting Assumption IV B. Order of determination: wings→ocelli→wing muscle. Change from wingless to winged. Normal wing muscle.

Slide No.	Characters to which Assumption IV B is fitted			Number of sensoria	Antennal color
	Wing length	Ocellar development	Wing muscle development		
1168 a.....	.60	.71	.83	5-8	0
472 c (12).....	.60	.71	.74		0
1061 e (1).....	.50	.94	.99	14-15	4
307 a (10).....	.50	.59	.74	11-11	3
472 c (3).....	.40	.46	.90		0
1196 c (4).....	.40	.46	.66	9-8	1
597 d (6).....	.35	.46	.83	6-6	0
1196 c (1).....	.30	.46	.58	6-6	0
44 c (2).....	.25	.46	.49	7-7	1
1077 a.....	.25	.71	.74	5-6	0
905 b (3).....	.25	.35	.45	5-8	0
1063 g (2).....	.22	.46	.66		1
414 c.....	.20	.35	.63	6-6	0
472 c (1).....	.20	.46	.57		0
1087 g (1).....	.20	.46	.62	5-6	0
1174 c.....	.12	.35	.41	5-5	0
1097 f.....	.10	.71	.99	12-9	3
717 b.....	.10	.59	.70	7-7	3
1015 f.....	.10	.59	1.00	11-7	0
472 c (4).....	.09	.35	.66		0
44 c (3).....	.08	.35	.46	7-8	1
472 c (8).....	.05	.35	.49		0
895 d.....	.04	.23	.37	6-8	0
267 d.....	.04	.11	.23	6-6	0
597 d (1).....	.04	.11	.33	6-6	0
472 c (14).....	.03	.35	.42		0
589 d (3).....	.03	.23	.42	6-7	1
44 c (1).....	.03	.23	.42	4-4	1
44 c (4).....	.02	.35	.40	8-8	1
589 d (4).....	.02	.33	.62	7-5	0
597 d (2).....	.02	.23	.33	6 or 7	0
569 d (3).....	.02	.23	.25		
295 c (2).....	.02	.11	.25	7-8	0
905 b (1).....	.02	.35	.49	6-7	1
569 e (1).....	.015	.23	.25		
1111 a (1).....	.01	.23	.33		1
1063 g (1).....	.01	.23	.41	5-5	0
1035 d (9).....	.01	.20	.33	6-7	0
1079 g (3).....	.01	.23	.37	5-5	0
1079 g (4).....	.01	.23	.37	6-6	0
1079 g (5).....	.01	.28	.41	5-5	0
1079 g (6).....	.01	.17	.37	5-7	0
1079 g (7).....	.01	.23	.28	4-7	0
1035 d (1).....	.01	.23	.28	5-7	0

TABLE VI (*continued*)*Composition of intermediate aphids fitting Assumption IV B*

Slide No.	Characters to which Assumption IV B is fitted			Number of sensoria	Antennal color
	Wing length	Ocellar development	Wing muscle development		
589 d (8).....	.01	.28	.37	7-6	0
589 d (7).....	.01	.23	.29	7-7	0
589 d (5).....	.01	.17	.29	8-7	1
1103 d (1).....	.01	.23	.37	6-6	0
1103 d (3).....	.01	.23	.37	5-5	0
1103 d (4).....	.01	.23	.33	5-5	0
1103 d (5).....	.01	.35	.49	4-4	0
1087 g (2).....	.01	.23	.41	5-4	0
1085 d (8).....	.01—	.11	.37		0
1035 d (6).....	.01—	.23	.33	3-5	0
1035 d (7).....	.01—	.23	.33	4-6	0
1035 d (5).....	.01—	.11	.23	8-5	0
307 a (7).....	0	.46	.58	13	4
773 b.....	0	.59	.74	8-9	2
308 a (4).....	0	.70	.72	12-13	3
454 b.....	0	.82	.91		
307 a (3).....	0	.58	.66	13	4

TABLE VII

Composition of intermediate aphids fitting Assumption V A. Order of determination: ocelli→wings→wing muscle. Change from winged to wingless. Degenerate wing muscle.

Slide No.	Characters to which Assumption V A is fitted			Number of sensoria	Antennal color
	Ocellar development	Wing length	Wing muscle development		
1017 c.....	.94	.45	.33	5-9	
224 c.....	.35	.30	.01—	6-7	0
227 d.....	.35	.20	.01—	6-7	1
1162 c.....	.59	.20	.01—	6-6	0
44 c (6).....	.23	.15	.01—	5-7	1
287 e (1).....	.35	.15	.01—		
44 c (5).....	.23	.10	.08	5-7	0
219 c.....	.46	.10	.01—	5-5	0
228 e.....	.35	.10	.01—		
295 d (1).....	.23	.06	.01—	6-6	0
296 b (2).....	.23	.05	.03	6-6	0
40 c (3).....	.23	.05	.04	6-6	0
270 e (1).....	.11	.03	.01—	6-7	0
295 c (3).....	.11	.02	.01—	6-7	2
887 d.....	.35	.02	.01—	6-5	0

TABLE VIII

Composition of intermediate aphids fitting Assumption V B. Order of determination: ocelli→wings→wing muscle. Change from wingless to winged. Normal wing muscle.

Slide No.	Characters to which Assumption V B is fitted			Number of sensoria	Antennal color
	Ocellar development	Wing length	Wing muscle development		
1091 d (1).....	.47	.70	.83	7-7	1
1091 d (2).....	.47	.70	.86	9-9	1
589 d (2).....	.59	.60	.67	9-10	1
1089 g.....	.33	.45	.58	7-11	0
597 d (5).....	.33	.40	.49	5-4	0

TABLE IX

Composition of intermediate aphids not fitting any time-of-determination assumption. Degenerate wing muscle.

Slide No.	Characters to which no Assumption is fitted			Number of sensoria	Antennal color
	Wing length	Ocellar development	Wing muscle development		
214 c.	.40	.40	.33	5-6	0
490 g (3).....	.01—	.23	.01—		
P-39.....	.01—	.11	.01—		
490 g (2).....	.01—	.23	.01—		

of the several organs is concerned. For example, aphids conforming to Assumption III B show the same composition as those conforming to Assumption IV A; they differ only in the direction of change as established by the histological character of the wing muscle. In Assumption III B, the change is from wingless to winged; in Assumption IV A it is from winged to wingless. If degenerate muscles are considered the result of a change from winged to wingless, an individual with a given composition fitting equally well both Assumptions III B and IV A would be placed in IV A if the muscles were degenerate. There is one outstanding piece of evidence that degenerate wing muscles do indicate a transition from winged to wingless aphids. Comparing the first few aphids tabulated in Tables V and VI, it will be observed that the wing development is what one would expect if the wings were determined first as here proposed. At the top of Table V (winged to wingless) is listed an intermediate with fully developed wings, which is possible if wings are determined before wing muscles, but could not

occur if muscles were determined before wings. On the other hand, the first aphid listed in Table VI (wingless to winged) shows only .6 of normal wing length. It is probably not without significance that the first few aphids listed in Table V show considerably more wing development than the first few listed in Table VI. If wings are determined first, then in a winged-to-wingless transition the wings might be expected to show on the whole more development than in a wingless-to-winged change. The other possibility would be to postulate that wings are determined last, which does not fit the facts, as such an hypothesis would rule out the combination of fully developed wings and reduced wing muscle. More than that it is hardly conceivable even in an aphid with the composition shown by No. 285 e (2), Table V, wing length .8, ocellar development .59, and wing muscle development .12, that wing muscle could have been determined first, which the alternative theory would require. Thus all available evidence points to the conclusion that wings are determined before wing muscle, and that degenerate wing muscle characterizes a transformation from a winged to a wingless aphid in development.

So far in this paper no attempt has been made to prove that the ocelli are segregated at a time between the wings and wing muscles. Some evidence for this position of the ocelli in the order of determination is available. To arrive at this evidence it will be helpful to show what would occur in the event that ocelli were determined between wing and wing muscles at an arbitrarily set time. In the chart below it is assumed that the order of determination is wings, ocelli, and wing muscle. There is also the assumption made that no overlapping in times of segregation for the several organs occurs. The times indicated are purely hypothetical as nothing is known about the actual time involved.

A.

<i>Change from winged to wingless</i>		
Wing determination	Ocellar determination	Wing muscle determination
1:00 o'clock.....	2:30 o'clock. Change. to wingless	3:00 o'clock
Fully developed.....	One-half developed	No development

B.

<i>Change from wingless to winged</i>		
Wing determination	Ocellar determination	Wing muscle determination
1:00 o'clock.....	2:30 o'clock. Change to winged	3:00 o'clock
No development.....	One-half developed	Fully developed

The ocelli in *A* are only one-half developed because a change in physiology unfavorable to their development took place when they were only half segregated in the embryogeny. In *B* the ocelli are only one-half developed because a change in physiology which made their development possible in the embryogeny did not occur until the period of determination was half completed.

From this illustration it is evident that under the conditions postulated, the ocelli may not be very different in amount of development in two aphids derived from opposite directions of change, but the wings and wing muscles would show a considerable difference. If again Tables V and VI are compared it will be found that the facts conform to the theory. For example, Table V, slide 285 e (2), wing length .80, ocellar development .59, wing muscle development .12, compared with Table VI, slide 307 a (10), wing length .50, ocellar development .59, wing muscle development .74, shows exactly the same relationship as illustrated above. Not all of the aphids fit the conditions as perfectly as the ones drawn for illustration but the general trend is clearly the same.

Thus the facts support the theory that of the three organs, wings, ocelli, and wing muscle, the ocelli are median in the time of embryonic segregation.

If the time-of-determination theory were perfectly applicable to intermediate-winged aphids, and the intermediates were to be classified according to the assumptions of Tables I to IX inclusive, which are based on the time-of-determination theory and the direction of change established by the character of the muscle, then all intermediates would meet the conditions which parts "*A*" and "*B*" of one assumption entail. Examination of Tables I to IX, which are an attempt to discover the applicability of the time-of-determination theory when the foregoing conditions are imposed, shows that the material does not all fit any one assumption. However, Assumption IV is applicable to about 69 per cent of the aphids of this research. Other assumptions which are applicable to intermediates are I (2 per cent), II (10 per cent), and V (16 per cent). This would suggest that the time-of-determination theory has at least a limited applicability to the development of intermediate-winged aphids. In fact, the large percentage conforming to this scheme probably means that the assumption that the composition of intermediates is determined by the times of embryonic segregation of their differential structures is in a general way correct. Were there no such relation between the organs of intermediacy a much smaller proportion of intermediates should be of the composition shown in Tables V and VI. As previously stated, there are 6 conceivable assumptions

for these intermediates. If the combinations were entered into at random only one-sixth of them should be of the kinds listed in Assumption IV (Tables V and VI). Their actual numbers are over four times this expectation.

Besides the 84 intermediates which conform to a single assumption regarding times of determination (Tables V and VI), there are 34 which fit other assumptions (Tables I, II, III, IV, VII, and VIII), and 4 that could not be classified under any one of these schemes (Table IX). It will be noted concerning these aphids not meeting the conditions of Assumption IV that most of them have been placed in another assumption due to the fact that they appear to show a shift in the time of determination of the ocelli. In most instances the development indicates a determination of ocelli before wings, while in two cases the amount of ocellar development indicates their determination after the wing muscles. This variation of the indicated time of determination of the ocelli in both directions suggests again that the usual time of segregation is between the wings and wing muscles.

The simplest explanation of the irregularities in the order of determination found in Tables I, II, III, IV, VII, and VIII, after eliminating certain possible errors, is to postulate that there actually is variation in the time at which segregation takes place. The variation on at least a few occasions is so great that the time when the wings are determined is changed from the first of the three to the last. However, of particular interest is the fact that in all of the material studied not one case was found in which wing muscles were indicated to be determined first. It may be significant that there are far more individuals in which there was a change in the usual time of determination of only two structures as contrasted with those in which the position of all three was disturbed. For it would seem reasonable if this explanation is correct that slight irregularities would occur more frequently than those which involved more radical departures from the usual. The small group of four aphids (Table IX), in which two organs are of about the same development are exceptional individuals in which a change in the time of segregation of only one structure may have caused the irregularity. That the time of determination is not an unalterable fixed thing is shown not only by these irregular classifications, but in Tables V and VI, to which the intermediate group as a whole best conforms, there is considerable variation among the individuals listed.

AN INTERPRETATION OF THE COMBINATIONS OF CHARACTERS IN
INTERMEDIATE APHIDS BASED ON THE PHYSIOLOGICAL LEVEL THEORY

Inasmuch as the combinations of characters in the intermediate aphids of this report do not conform perfectly to the time-of-determination theory, an analysis based on the physiological level theory would seem indicated. It will be remembered from an earlier statement that this theory postulates that the degree of intermediacy depends upon the state of some physiological process during development. It could depend on the quantity of some substance (possibly a hormone) present during the embryogeny. If all the differentiating structures reacted to exactly the same extent to a given quantity of this substance, or to the "level" of this physiological process, there would not appear to be a linear order of determination; all structures would give evidence of having been determined at the same time, or during the same part of the embryogeny. According to this theory without modification, when a given degree of intermediacy in one structure occurs, each other organ should show a definite degree of intermediacy. For example, if one intermediate at a given physiological level shows .1 wing, .2 ocellar, and .4 wing muscle development, every intermediate with .1 wing development should have the same grade of ocellar and wing muscle development. If, however, wings start development at a given physiological level, let us say when there is a certain concentration of a substance, while ocelli and wing muscles do not, there arises a situation in which a difference in the time of determination of these organs is surmised, although such a difference may not exist. In other words, there may be a difference in the threshold of stimulation of the intermediate structures causing their appearance in different degrees. This explanation denies any importance to differences in the time of embryonic segregation of differentiating parts. There could be such differences even if there were a difference in the threshold of stimulation of the several organs, but it would not be the differences in time of determination of the different parts that would determine the composition of intermediate individuals, rather it would be the differences in the threshold of stimulation of the differentiating parts.

A casual examination of the tables of intermediate-winged aphid data indicates at once that the physiological level theory without modifications is not applicable to these aphids, for there is apparently no constant relationship between the degrees of development of the several structures such as this theory requires. But if it is assumed that there is a difference in the threshold of stimulation of the several organs in dif-

ferent individuals then there should be a difference in the development of a given structure in different intermediates. If Tables V and VI, which include nearly 70 per cent of the material of this investigation, are studied, it will be found, to take a typical example from Table V, slide 1196 c (11), that wings are .6, ocelli .41, wing muscle .23 in development. This would mean, according to the physiological level hypothesis, that the wings possessed a lower threshold of stimulation than either of the other two structures. Now if a comparison is made with Table VI, slide 1168 a, in which the wings are .6, ocelli .71, and wing muscle .83 developed, it will be seen that in this case the wing muscle would have to possess the lowest threshold of stimulation, ocelli the next lowest, and the wings the highest. It will be noted in the latter illustration there is required a reversal of the order of relative levels in the thresholds of stimulation of the several structures; the threshold of stimulation of the wings in the second illustration is highest, whereas in the first illustration it was lowest. Thus, if this proposal of a varying threshold of stimulation is offered as a modification of the physiological level hypothesis in explanation of the combination of characters in the majority of intermediates in this work, it is necessary to postulate not only that the threshold of stimulation is different in different organs for the same individual, but that it is different for the same organ in different individuals, and that for most intermediates there is an alternating though definite order in which the threshold of stimulation varies. Other aphids which do not conform to Tables V and VI would necessitate the assumption of an order in which the thresholds of stimulation in the several organs vary. It is conceivable that the thresholds of stimulation could vary for the several structures in different aphids in such a manner as to produce a series fitting the time-of-determination scheme, but it seems highly improbable that at one time the threshold would so vary as to give individuals of a composition that would conform to the winged-to-wingless direction of change and at another time individuals of such a composition that they would fit a wingless-to-winged direction of change. The difficulties involved in making this assumption provide the greatest objection to this hypothesis.

DISCUSSION

With the exception of the work of Shull on aphids, most of the previous investigations concerning intermediacy in insects have dealt with intersexes, that is, forms intermediate between the male and female of the species. The chief contributor in this field has been Goldschmidt (1931), who, in an analysis of intersexuality of the gipsy moth, *Ly-*

mantria dispar, considers intersexuality in these moths as depending chiefly on general sex genes.

The intermediacy of aphids which has been a subject of considerable study at the University of Michigan is in sharp contrast in some respects to that of the moth reported by Goldschmidt. In the first place, the type of intermediacy which we have studied in aphids is not an intersexual phenomena, for we are dealing with only one sex. Secondly, it is highly improbable that it is due to a quantitative genic influence as postulated for *Lymantria*, for the aphids of this research were all parthenogenetic females, and in a parthenogenetic line there is a minimum of opportunity for genetical variation. Certainly the differences in individuals of a single strain of a parthenogenetic species are less likely to result from genetical causes than in an animal which reproduces bisexually as in the gipsy moth where different geographical races were involved in the crosses. Inasmuch as it has been found possible by modification of the environment to change the percentage of winged to wingless aphids, it would seem probable that intermediacy was due to some physiological state induced by environmental factors. However, Goldschmidt's assumption that embryonic determinations occur at different times in development does with modifications seem applicable to aphids, as has been discussed above. Modifications from a perfect application of the time-of-determination theory to aphids are necessary because the data from intermediate-winged aphids not only show considerable variation in the times of determination within the usual order of embryonic segregation but this sometimes is disturbed by irregularities.

Thus it will be seen that in two types of intermediacy which are quite different in that one is of an intersexual character and results from a genic imbalance, the other not intersexual and probably due to environmental factors, yet both support in a general way the theory that in at least some of the invertebrates there is a succession of embryonic determinations.

SUMMARY

In two strains of aphids of the species *Macrosiphum solanifolii*, intermediates between winged and wingless females were experimentally produced, and a histological study was made of these. Aphids may show internal intermediacy when there is no evidence of it externally, therefore the degree of winged-wingless intermediacy cannot be safely judged by external observations alone.

Two theories attempting to explain the combination of characters (wings, ocelli, wing muscle) found in intermediate-winged aphids are

discussed. The first theory postulates that the combination of characters in intermediates is due to the fact that the differentiating organs are determined at different times in the embryogeny (time-of-determination theory), while the second theory assumes that the structural character of intermediates results from a given concentration of some substance (physiological level theory) in development.

It could not be ascertained from the conditions under which these aphids were reared what direction of change (winged to wingless or wingless to winged), assuming there was a change, produced a given winged-wingless intermediate, but evidence is given which indicates that the character of the wing muscle is correlated with some definite change in the direction of embryonic development. The evidence makes it appear probable that degenerate wing muscle means that the intermediate aphid is the result of some physiological change from a winged to wingless form during the embryogeny; whereas the intermediate with normal wing muscle appears to be the result of a change from wingless to winged during its development.

If the time-of-determination theory were perfectly applicable to intermediate-winged aphids they should all fit one (the same one) of the six possible assumptions for the time at which the wings, ocelli and wing muscles may be determined in the embryogeny. More than two-thirds of the aphids of this study did fit one assumption (Assumption IV) which is evidence that in a general way the composition of intermediates is determined by the segregation of their differentiating structures at different times in the embryogeny. This indicates that organs are determined in a serial order in aphids, as has been found true for certain vertebrates. The order of embryonic determination of the structures distinguishing winged from wingless is wings, ocelli, and wing muscles.

The small differences between the amount of development in wings, ocelli, and wing muscles found in many cases is taken to indicate that the time of determination of these organs is close together. However, that the period of embryonic segregation for these three structures is of considerable duration is suggested by the large number of intergrades.

Most of the intermediates which did not conform to Assumption IV appeared to owe their irregular combination of characters to a shift in the time of determination of the ocelli. The simplest explanation of these irregularities is to postulate that the time of segregation may vary for a given organ in different individuals.

An interpretation of the combination of differentiating characters of intermediate-winged aphids based on the physiological level theory would involve the necessity of assuming that the threshold of stimulation

for the development of these organs varies in different individuals. Moreover, the threshold of stimulation would have to show a series of regular variations to produce aphids fitting one direction of development, as winged to wingless, at one time and another direction, as wingless to winged, at another time. The difficulties involved in making such an assumption renders this hypothesis highly improbable.

The results of this investigation support the time-of-determination theory with the modification of frequent variations in the time at which an organ may be embryonically determined.

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